Hemoglobin cut-off values in healthy Turkish infants

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Background: **Anemia is a widespread public health problem associated with an increased risk of morbidity and mortality. This study was undertaken to determine the cut-off value of hemoglobin for infant anemia.**

Methods: **A cross-sectional retrospective study was carried out at well-baby clinics of a tertiary care hospital. A total of 1484 healthy infants aged between 4 to 24 months were included in the study. The relationship of hemoglobin (Hb) levels with mother age, birth weight, weight gain rate, feeding, and gender was evaluated.**

Results: **The Hb levels were assessed in four age groups (4 months, 6 months, 9-12 months, and 15-24 months) and the cut-off values of Hb were determined. Hb cut-off values (5th percentile for age) were detected as 97 g/L and 93 g/L at 4 months and 6 months, respectively. In older infants, the 5th percentile was 90.5 g/L and 93.4 g/L at 9-12 months and 15-24 months, respectively. The two values were lower than the World Health Organization criteria for anemia, which could partly due to the lack of information on iron status in our population. However, this difference highlights the need for further studies on normal Hb levels in healthy infants in developing countries. Hb levels of females were higher in all age groups; however, a statistically significant difference was found in gender in only 6 month-old infants. No statistically significant difference was found among Hb levels, mother's age, birth weight, weight gain rate, and nutritional status.**

Conclusion: **Hb cut-off values in infants should be re-evaluated and be compatible with growth and development of children in that community.**

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Introduction

Anemia is a widespread problem associated with an increased risk of morbidity and mortality. The World Health Organization (WHO) estimates that more than 2 billion people are anemic worldwide. Anemia in infants (approximately 50% of anemic cases are attributable to iron deficiency) is causally associated with poor growth, poor development, reduced immunity, cognitive impairment and decreased physical capacity.[1] The prevalence of iron deficiency anemia (IDA) in infants in Turkey varies from 2% to 46%, whereas the prevalences of alpha and beta thalassemia traits are 2.9% and 2.1%, respectively.[2,3] Anemia is defined as a hemoglobin (Hb) level below an established cut-off value. Based on the WHO criteria, children aged 0.5-5 years with a Hb level less than 110 g/L are considered to be anemic.[4] The WHO recommended cut-off value covering wide age range can't accurately reflect the prevalence of anemia in children (especially in infants) reported in several studies.[5-8] Thus, the WHO report in 2001 indicates a difference of 5-10 g/L in mean Hb values between European and African children.[9] Several studies[4-10] have suggested alternative Hb cut-off values for infants and young children. Two studies[6,7] on British infants reported that cut-off values of Hb for anemia in infants aged 8 months were 97 g/L whereas they tended to be 100 g/L for 12-18 months old infants. However, some other studies[8,9] determined that the Hb cut-off value of 100 g/L for infants aged 9 months was more appropriate, and they declared that the cut-off value recommended by the WHO is not applicable for all children. There is a need to define more appropriate and population specific cut-off values for anemia in infants and young children. However, there have been a limited number of studies addressing gender difference of mean Hb values in healthy infants.[6-8,10]

The current study aimed to detect the distribution of Hb values in healthy Turkish infants, to establish reference percentiles for Hb, and to analyze the changes in Hb values by some parameters.

Methods

Design and setting

The study was approved by the Ethics Committee of the Cerrahpasa Faculty of Medicine of Istanbul University.

Original article

The medical files of 2550 infants at 0-24 months of age who were followed up at Children's Hospital Well Child Clinic, Cerrahpasa Medicine Faculty of Istanbul University between January 2005 and December 2007 were evaluated retrospectively. The infants attending this clinic had different socioeconomic status, and the study population was a representative sample of the general population. Premature infants, infants who had previous or current iron supplementation, infants with acute or chronic illnesses, growth failure during follow-up period, a familial history of thalassemia or a history of exchange transfusion, intrauterine growth retardation and hydrops fetalis were excluded. Altogether 1484 healthy infants were eligible for this study.

In our clinic, complete blood cell count was detected as a routine screening between 4 and 24 months (usually at 4 or 6 months of age) for each infant during a follow-up examination. In developing countries, where the prevalence of IDA is high, the screening time is appropriate. The reason of this routine screening is to diagnose iron deficiency anemia. Each infant in the study population had only one Hb value (one measurement per infant). Complete blood count, Hb, hematocrit, mean corpuscular volume (MCV), mean corpuscular Hb, and red cell distribution width (RDW) were measured by an automated analyser (Sysmex XT- 2000i™; Roche Diagnostics, Kobe, Hyogo, Japan). Age of infants, gender, mother's age, nutritional and breast feeding status, birth weight and monthly weight gain rate were also recorded. According to their nutritional status at 4 months of age, the infants were divided into three groups: exclusively breast-fed, exclusively formula-fed (iron-fortified infant formulas, iron contents with 1-1.5 mg/100 kcal) and mixed-fed infants. Hb values were also analyzed in four groups: 4, 6, 9-12 and 15-24 months of age. The 5th percentile of Hb was defined as the cut-off point for anemia.

Statistical analyses were performed using SPSS software (version 16.0, SPSS Inc, Chicago, IL, USA). The analyses were made between the groups. The association between Hb values and independent variables in the groups (gender, monthly weight gain, mother age and nutrition) was analyzed using descriptive statistics. The relationship between Hb and gender was evaluated by Student's *t* test. The relationship between Hb and mother age and between Hb and nutrition in the 4 months old group and 6 months old group was evaluated by one-way ANOVA, but in the 9-12 months old group and 15-24 months old group was analyzed by the Kruskal-Wallis test because the number of children in these groups was appropriate for non-parametric test. The relationship between monthly weight gain and Hb value was evaluated by linear regression analysis. A P value <0.05 was considered statistically significant.

Results

The mean age of children was 6.13±3.71 months, and 47.2% of the children were female. The mean birth weight of the children was 3318±450 g (2500-4980 g). Exclusive breast-feeding rate for 4 months was 81.5%, whereas exclusive formula-feeding rate was 3.1% and mix-feeding rate was 15.4%. The mean maternal age was 26±5 years (17-48 years). Adolescent mother (15-19 years) rate was 1.5%, and the old maternal age (36-48 years) rate was 11.7%. Fifteen percent of the mothers were elementary school graduates (5 years), while 74% of the mothers were secondary school graduates (8 years). Some descriptive and analytic characteristics of the study population are shown in Table 1.

The Hb values of girls were higher in all age groups; however, a statistically significant difference was found in gender was found only in 6 months old infants. Boys in this group had a Hb value lower than girls (107.1±8.5 g/L *vs.* 109.7±8.1 g/L, P<0.0001). In infants of all groups, no statistically significant difference was observed between Hb levels, mother's age, birth weight, weight gain rate and nutritional status in the first 4 months (Table 1). The cut-off Hb values (5th percentile) in the infants of the four groups were 97 g/L, 93 g/L, 90.5 g/L and 93.4 g/L, respectively. The Hb percentile values in all groups are shown in Table 2. Hb percentile values in 4 and 6 months old groups are shown in Figs. 1 and 2.

Discussion

The observed Hb values of the infants in the present study were lower than the threshold of 110 g/L recommended by the WHO. Reference data are based on predominantly formula-fed infants. Morever, these values are extrapolated from older age groups, and may not be appropriate for infants. Yet, a number of previous studies from different parts of the world have shown that the WHO cut-off value for anemia is higher than the detected Hb cut-off value.[7-10] Two studies conducted on British infants at 8 months of age and 12-18 months of age reported that the cut-off values based on the 5th percentile of Hb values were 97 g/L and 100 g/L, respectively.[6,7] Another survey also reported lower Hb cut-off values compared to the WHO criteria.[11] A study on Estonian infants revealed that the Hb cut-off value using the 5th percentile at age of 9-12 months was 101 g/L, higher than that observed in our infants in the same age group.[9] We assumed that the differences of Hb value in these studies are associated with nutritional

Table 1. Descriptive and analytic characteristics of the age groups

	4 mon	6 mon	9-12 mon	15-24 mon
Sex (*n*)				
F	235 (50.2%)	330 (46.9%)	112 (48.9%)	46 (55.4%)
M	233 (49.8%)	374 (53.1%)	117 (51.1%)	37 (44.6%)
total	468	704	229	83
Hb (F)	11.1±0.75	10.9±0.81	10.8±0.85	11.4±0.99
Hb (M)	11.0±0.93	10.7±0.85	10.6±0.93	11.0±0.96
Hb-sex association	*P*=0.304	*P*<0.0001	*P*=0.115	*P*=0.085
Birth weight (g)* (range)	3278±447 (2500-4890)	3352±450 (2500-4950)	3329±440 (2500-4740)	3406±466 (2500-4980)
Hb-birth weight correlation	*P*=0.408	*P*=0.565	*P*=0.187	*P*=0.210
Weight gain† (range)	939±76 (265-1575)	787±81 (140-1230)	611±111 (377-983)	444±84 (274-641)
Hb-weight correlation	*P*=0.219	*P*=0.347	*P*=0.970	*P*=0.85
Mother age (y)				
<20	4 (0.9%)	16 (2.3%)	2 (0.9%)	1 (1.2%)
20-34	395 (84.4%)	615 (87.3%)	207 (90.4%)	71 (85.5%)
>35	69 (14.7%)	73 (10.4%)	20 (8.7%)	11 (13.3%)
Hb-age correlation	*P*=0.111	*P*=0.678	*P*=0.114	*P*=0.330
Nutrition (0-4 mon)				
Breast milk	403 (86.1%)	529 (75.1%)	185 (80.8%)	70 (84.3%)
Formula	13 (2.8%)	34 (4.8%)	3 (1.3%)	12 (14.5%)
Mixt	52 (11.1%)	141 (20%)	41 (17.9%)	1 (1.2%)
Hb-nutrition correlation	*P*=0.553	*P*=0.525	*P*=0.415	*P*=0.055

*mean±SD; †: g/mon, mean±SD. F: female; M: male; Hb: hemoglobin.

Table 2. Hemoglobin percentiles (g/L) in 4, 6, 9-12 and 15-24 months old children

P	4 mon			6 mon			9-12 mon			15-24 mon		
	Girls	Boys	Total	Girls	Boys	Total	Girls	Boys	Total	Girls	Boys	Total
n	235	233	468	330	374	704	112	117	229	46	37	83
5 th	98	97	97	96	92	93	92	92	90	91	93	93
10 th	103	99	100	100	96	97	98	93	96	102	96	98
25 th	107	104	106	105	102	103	102	100	101	109	105	107
50 th	111	111	111	110	108	109	109	107	108	114	111	112
75 th	116	116	116	115	114	114	114	114	113	120	117	119
90 th	120	122	121	119	118	118	118	118	118	125	124	124
95 th	124	126	125	122	121	122	122	122	120	130	127	127
Mean	111	110	111	109	107	108	108	106	107	114	110	112
SD	7	9	8	8	8	8	8	9	9	10	9	9
MCV (m*, fL)	78.6	77.6	78.1	75.3	73.1	74.1	73.0	72.4	72.3	75.6	73.4	74.6
SD	4.5	4.8	4.7	4.1	5.1	4.8	5.2	5.2	5.2	5.3	5.7	5.6
n†	228	216	444	307	346	653	103	110	213	40	43	73
RDW (m, %)	11.9	12.1	12.0	12.9	13.4	13.2	14.1	14.3	14.2	14.1	14.2	14.1
SD	1.3	1.2	1.3	1.5	1.6	1.6	1.6	1.6	1.6	1.9	2.0	1.9
n†	166	161	327	228	251	479	63	80	143	26	24	50
RBC (m, mm^3)	4.1	4.2	4.1	4.2	4.2	4.2	4.3	4.2	4.3	4.4	4.5	4.5
SD	0.3	0.4	0.4	0.3	0.4	0.4	0.5	0.5	0.5	0.2	0.5	0.4
n†	102	97	199	117	127	244	35	40	75	10	12	22

*: mean value; †: the number of subject population is missing because missing data in children's files. MCV: mean corpuscular volume; RDW: red cell distribution wave; RBC: red blood count.

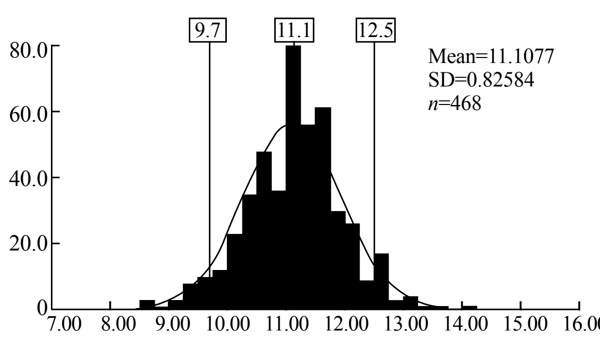

Fig. 1. Hb curves (mean, 5 and 95 percentiles) in 4 months age group.

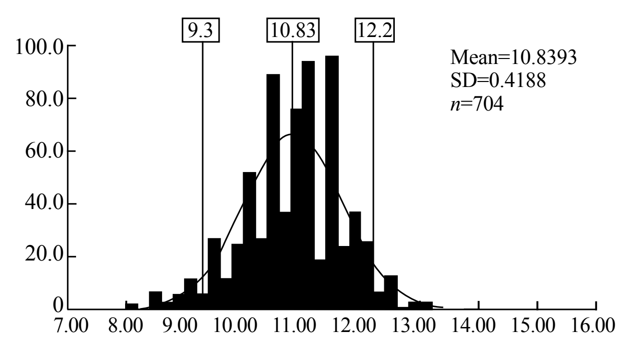

Fig. 2. Hb curves (mean, 5 and 95 percentiles) in 6 months age group.

status, maternal age, birth weight, and weight gain of infants. In Swedish and Honduran infants who were exclusively breastfed for six months and received no iron supplementation, Domellöf et al[12] found different Hb cut-off levels. The Hb level was 105 g/L in Swedish infants and 96 g/L in Honduran infants at 4 months of age, but it was 105 g/L at 6 months of age, and 100 g/L at 9 months of age in Swedish infants with sex indifference. In the present study, the Hb cut-off levels were lower than those in Swedish infants, but higher in Honduran infants. It is clear that these Hb values are quite lower than the WHO cut-off value. Differentiation of normal Hb values of healthy infants in different communities may be up to genetic or environmental factors. Alpha thalassemia is not one of the most common genetic causes for lower Hb level, because its prevalence in Turkey is lower than most of the Middle East and Mediterranian countries.

Hb cut-off levels different from the WHO criteria are also shown in the literatures as source.[11-15] Our results cannot be directly compared with other Hb levels because the above analyses included a wide age range. A study on 9 month-old African-American infants found a prevalence of anemia of 25.3%, 10.1% or 1.5% at Hb cut-offs <110 g/L, 105 g/L, or 100 g/L, respectively.[16] Michaelsen et al[17] also found the prevalence of anemia in infants was different according to the Hb cut-off levels. The studies mentioned above showed that small changes of Hb cut-off levels lead to major differences in the prevalence of anemia. The same Hb limit values have also been used in infants. Vendt et al[8] observed that sex affected the mean Hb value and MCV and that Hb level in boy infants at 9 months of age was 4g/L lower than in girl infants. They concluded that some of the differences may be genetically determined, but others thoutht that these differences may be related to faster growth velocity in boys, and hence a larger need for iron.[8,18] In the current study, Hb values in female infants were higher than those in male infants. However, the differences between both sexes were statistically significant only in the 6 months old group.

Previous studies reported that Hb values and serum iron levels of exclusively breast-fed infants *vs.* formula-fed infants for the first 6 months were not significantly different.[19-21] However, there are conflicting data about the prevalence of anemia in breast-fed infants in 6 months and over.[22,23] A study[24] showed that 20% of infants breast-fed for 190 days were found to be anemic, whereas another study[25] found that the prevalence of anemia in infants exclusively breast-fed for 9 months was 28%. In the current study, the prevalence of anemia was not found in infancy. However, Hb level was not related to nutritional status in infants of 4-6 months old. Exclusive breast-feeding during the first 6 months could reduce iron deficiency and iron deficiency anemia compared to the early introduction of complementary foods. In the complementary feeding period (≥6 months), the rates of formula feeding and cow milk feeding in infants were fairly low. However, traditional complementary foods of our infants (vegetable purees, cereals, yoghurt, etc) are not iron rich. Also, no significant difference was found between Hb level, birth weight and weight gain. Hb and iron levels of mothers did not affect their babies despite the contradictory results of some studies. Iron deficiency in pregnancy may decrease the Hb level of infants.[26,27] We cannot confirm the relationship between Hb levels of mothers and and their infants because of the absence of maternal Hb levels. In the present study, no association was observed between maternal age and Hb levels.

The number of infants of 9-12 months old and 15-24 months old was lower than that of younger infants (*n*=229, *n*=83, respectively). Therefore, the number of these infants was not considered satisfactory for the measurement of Hb cut-off values. Also, the 5th percentile was 90.5 g/L and 93.4 g/L at 9-12 months and 15-24 months, respectively. The two values which were lower than the WHO criteria could partly due to the lack of information on iron status in our population. However, this difference highlights the need for further studies of Hb levels in healthy infants in developing countries.

In our study, serum ferritin and iron levels were not measured. However, MCV values in infancy age groups were not under 70 fl, and RDW and RBC were normal (12-14%, $>4\times10^6$/mm3, respectively). Growth and development of these babies were normal.

In conclusion, more appropriate Hb cut-off values are needed to define anemia in infants and children, and the data of the current study must be interpreted with caution because the WHO cut-off value (<110 g/L) may be higher for anemia diagnosis in healthy infants in developing countries. We suggest that these Hb values at 4 and 6 months of age would be a reference for detecting iron deficiency anemia in infants in these countries. The reference Hb cut-off values should be appropriate with age, nutritional status and growth of community dwelling children. Therefore, further prospective research is needed to clarify these associations.

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protocol development, patient screening, enrollment, outcome assessment, preliminary data analysis and writing the manuscript. Gür E participated in the development of the protocol, analytical framework for the study, data analysis and writing the manuscript.

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